Plant and Soil (2005) 274:175–195
DOI 10.1007/s11104-004-1158-7

The physiology, genetics and molecular biology of plant aluminum resistance and toxicity

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Received 7 January 2004. Accepted in revised form 5 February 2004

Key words: aluminum resistance, aluminum toxicity, anion channel, organic acid exudation

Abstract

Aluminum (Al) toxicity is the primary factor limiting crop production on acidic soils (pH values of 5 or below), and because 50% of the world's potentially arable lands are acidic, Al toxicity is a very important limitation to worldwide crop production. This review examines our current understanding of mechanisms of Al toxicity, as well as the physiological, genetic and molecular basis for Al resistance. Al resistance can be achieved by mechanisms that facilitate Al exclusion from the root apex (Al exclusion) and/or by mechanisms that confer the ability of plants to tolerate Al in the plant symplasm (Al tolerance). Compelling evidence has been presented in the literature for a resistance mechanism based on exclusion of Al due to Al-activated carboxylate release from the growing root tip. More recently, researchers have provided support for an additional Al-resistance mechanism involving internal detoxification of Al with carboxylate ligands (deprotonated organic acids) and the sequestration of the Al-carboxylate complexes in the vacuole. This is a field that is entering a phase of new discovery, as researchers are on the verge of identifying some of the genes that contribute to Al resistance in plants. The identification and characterization of Al resistance genes will not only greatly advance our understanding of Al-resistance mechanisms, but more importantly, will be the source of new molecular resources that researchers will use to develop improved crops better suited for cultivation on acid soils.

Introduction

Aluminum (Al) toxicity is the primary factor limiting crop production on strongly acidic soils. At soil pH values at or below 5, toxic forms of Al are solubilized into the soil solution, inhibiting root growth and function, and thus reducing crop yields. It has been estimated that over 50% of the world's potentially arable lands are acidic (von Uexküll and Mutert, 1995; Bot et.al., 2000); hence, Al toxicity is a very important worldwide limitation to crop production. Furthermore, up to 60% of the acid soils in the world occur in developing countries, where food production is critical. Breeding crops with increased Al resistance has been a successful and active area of research; however, the underlying molecular, genetic and physiological bases are still not well understood. Because of the agronomic importance of this problem, this is an area that has attracted significant interest from a number of molecular biology and physiology laboratories around the world. Despite the interest from many researchers, no Al resistance genes have yet been cloned from any plant. However, recent progress by a number of researchers has set the stage for the identification and characterization both of the genes and associated physiological mechanisms that contribute to Al resistance in important crop species grown on acid soils. This

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should, in turn, provide the necessary molecular tools to address a worldwide agronomic problem that is only exceeded by drought stress with regards to abiotic limitations to crop production (von Uexküll and Mutert, 1995).

Physiological mechanisms of aluminum toxicity

Aluminum in soils is present as insoluble alumino-silicates and oxides. As the soil pH drops below 5, the octahedral hexahydrate $Al(H_2O)_6^{3+}$, more commonly referred to as $A1^{3+}$, is solubilized into the soil solution. This form of Al appears to be the most important rhizotoxic Al species (Kinraide, 1991; Kin-raide and Parker, 1989, 1990). Al interferes with a wide range of physical and cellular processes. Potentially, Al toxicity could result from complex Al interactions with apoplastic (cell wall), plasma membrane, and symplastic (cytosol) targets. Given the vast literature and the diverse experimental approaches employed in studying Al toxicity, it is difficult to reach a consensus on the timing for Al toxicity as well as the cellular processes targeted by Al. For instance, while some Al-toxic symptoms and responses are detectable within seconds to minutes after exposure to Al, others are only discernible after long-term (hours to days) exposure. The direct association of long-term responses with mechanisms of Al toxicity should be interpreted cautiously as these may not be the result of a direct disturbance of a given pathway by Al, but could rather be the result of a general homeostasis disturbance on an unrelated physiological pathway triggered by an earlier Al-toxicity event.

Inhibition of root growth: The earliest Al-toxicity response.

Given the above, it is not surprising that a significant part of the research on Al toxicity has focused on the most rapid effects of Al on plant function. Root growth inhibition upon exposure to Al has been used extensively as a measurement of Al toxicity (Foy, 1988), as the primary and earliest symptom of Al toxicity is a rapid (beginning within minutes) inhibition of root growth (Kollmeier et al., 2000; Ryan et al., 1993; Sivaguru and Horst 1998; Sivaguru et al., 1999). Detailed spatial studies have indicated that within the root, the root apex, and more specifically the distal part of the transition zone within the apex, is the primary target of Al toxicity (Sivaguru and Horst, 1998). Within this root zone, recent studies have indicated that some Al can enter the cytosol of cells within minutes following Al exposure (Silva et al., 2000; Taylor et al., 2000; Vazquez et al., 1999). Consequently, although a large fraction of the Al interacts with apoplastic targets, a small fraction enters the symplasm and interacts with symplastic targets. The promptness of the root growth inhibition upon exposure to Al indicates that Al quickly disrupts root cell expansion and elongation, prior to inhibiting cell division (Frantzios et al., 2001; Wallace and Anderson, 1984). Prolonged exposures lead to Al interactions with the root cell nuclei, resulting in disruption of cell division and the cytoskeleton (Silva et al., 2000).

Al toxicity is associated with gross changes in root morphology (Ciamporova, 2002). Briefly, Al toxicity results in inhibited root elongation, which yields swollen root apices and poor or no root-hair development. This extensive root damage results in a reduced and damaged root system and limited water and mineral nutrient uptake (see, for example, Barcelo and Poschenrieder, 2002; Jones and Kochian, 1995). The degree of toxicity reported in the literature varies widely depending on the plant species, growth conditions, Al concentrations, and the duration of the exposure. Thus, given the complexity of the many cellular processes involved in root growth inhibition, the precise Al toxicity targets in this complex chain of events remain elusive.

Researchers have begun to dissect out and identify diverse Al targets in different pathways associated with root growth. Because Al is so reactive, there are many potential sites for injury, including: (A) the cell wall, (B) the plasma membrane, (C) signal-transduction pathways, (D) the root cytoskeleton, and (E) DNA/nuclei.

(A) The Cell Wall. X-ray microanalysis and secondary ion mass spectroanalysis have indicated that a significant fraction of Al in roots is associated with apoplastic binding sites, predominantly in walls of cells of the root periphery (Vazquez et al., 1999). The net negative charge of the cell wall determines its cation exchange

capacity (CEC), and consequently the degree to which Al interacts with the cell wall. Among the many components of the cell-wall network, pectins have been proposed to be a critical site for Al-cell-wall interactions (Blamley et al., 1993). Al interactions lead to the displacement of other cations (e.g., Ca^{2+}) fundamental for cell-wall stability (Mat-sumoto et al., 1977; Rincón and Gonzales, 1992; Schmohl and Horst, 2000; Tabuchi and Matsumoto, 2001), Consequently, the strong and rapid binding of Al can alter cell-wall structural and mechanical properties, making it more rigid, leading to a decrease in the mechanical extensibility of the cell wall required for normal cell expansion.

(B) The Plasma Membrane. Given its physicochemical properties, $A1^{3+}$ can interact strongly with the negatively charged plasma-membrane surface (Akeson and Munns, 1989; Kinraide et al., 1992, 1994, 1998). As Al has a more than 500-fold greater affinity for the choline head of phosphatildylcholine, a-lipid constituent of the plasma membrane, than other cations such as Ca^{2+} have, $A1^{3+}$ can displace other cations that may form bridges between the phospholipid head groups of the membrane bilayer (Akeson et al., 1989; Akeson and Munns, 1989). As a consequence, the phospholipid packing and fluidity of the membrane is altered. In addition, Al interactions with the plasma membrane lead to screening and neutralization of the charges at the surface of the plasma membrane that can alter the activities of ions near the plasma-membrane surface. Thus, Al interactions at the plasma membrane can modify the structure of the plasma membrane as well as the ionic environment near the surface of the cell; both can lead to disturbances of ion-transport processes, which can perturb cellular homeostasis.

Callose (β-l,3-glucane) synthesis (synthesized by β-l,3-glucane synthetase) on the plasma membrane is also quickly activated upon exposure to Al. Thus, callose accumulation in the apoplast has also been used as a measure of early symptoms of Al toxicity (Horst et al., 1997; Massot et al., 1999). Since callose synthesis depends on the presence of Ca^{2+}, it has been suggested that Al displacement of Ca^{2+} from the membrane surface may increase the apoplastic Ca^{2+} pool required to stimulate callose synthesis. Under Al stress, callose accumulation may lead to further cellular damage by inhibiting intercellular transport through plasmodesmatal connections (Sivaguru et al., 2000).

As mentioned above, Al binding to plasma membrane phospholipids surrounding transmembrane transporters may induce local charge disturbances, and alter local ion concentrations, thus affecting ion movement to binding sites in membrane-transport proteins. One of the most noticeable consequences of root Al exposure is an almost instantaneous depolarization of the plasma membrane (Lindberg et al., 1991; Papernik and Kochian, 1997). This change in the trans-plasma membrane electrochemical potential may be due to both direct and indirect interactions of Al with a number of different ion transport pathways (Miyasaka et al.,1989).

Plasma membrane H^+-ATPase. Al can significantly inhibit the activity of the plasma-membrane H^+-ATPase, impeding formation and maintenance of the trans-membrane H^+ gradient. Al-induced inhibition of H^+-ATPase activity and consequent disruption of the H^+ gradient has been reported both *in vitro* (e.g., membrane vesicle studies) and in intact roots of several plant species (Ahn et al., 2001, 2002; Ryan et al., 1992). The transmembrane H^+ gradient serves as the major driving force for secondary ion transport processes. Consequently, Al disruption of the H^+ gradient could indirectly alter the ionic status and ion homeostasis of root cells.

Inhibition of cation uptake and Al blockade of channel proteins. Exposure to Al can inhibit the uptake of many cations including Ca^{2+}, Mg^{2+}, K^+, and NH^+_4 (Huang et al., 1992; Lazof et al., 1994; Nichol et al., 1993; Rengel and Elliott, 1992; Ryan and Kochian, 1993). Although it has long been accepted that Al directly blocks root-cell ion transport proteins, it was not until fairly recently that evidence in support of such direct interactions has been presented. For example, a number of studies have shown that Al exposure strongly inhibits Ca^{2+} fluxes across the plasma membrane of root cells (Huang et al., 1992; Rengel and Elliott, 1992). Electrophysiological approaches were subsequently used to demonstrate that $A1^{3+}$ interacts directly with several different plasma-membrane channel proteins, blocking the uptake of ions such as K^+

and Ca^{2+} (Gassmann and Schroeder, 1994; Piñeros and Kochian, 2001; Piñeros and Tester, 1995). In addition to directly altering ion permeation through channels, extracellular Al can also modulate the transporter's activity via changes in the membrane potential. For example, Al-induced membrane depolarizations can alter voltage-dependent Ca^{2+} channel transport by indirectly modulating and shifting the activation thresholds of distinct transport pathways, such as hyperpolarization-activated (Kiegle et al., 2000; Very and Davies, 2000) and depolarization-activated (Piñeros and Tester, 1997; Thion et al., 1996; Thuleau et al., 1994) Ca^{2+} channels.

(C) Al Effects on Signal-Transduction Pathways.

Disruption of cytosolic Ca^{2+} and H^{+} activity. Al interactions with signal-transduction pathways, in particular disruption of intracellular Ca^{2+} and pH homeostasis, have been proposed to play crucial roles in Al toxicity. Several studies have shown that Al exposure can alter cytosolic Ca^{2+} and pH levels (Jones et al., 1998a, b; Lindberg and Strid, 1997; Ma et al., 2002b; Rengel, 1992; Zhang and Rengel, 1999). Al can also interact with and inhibit the enzyme phospholipase C of the phosphoinositide pathway associated with Ca^{2+} signaling (Jones and Kochian, 1995; Jones and Kochian, 1997). The Al-induced disruption of ion fluxes described above could directly lead to changes in cytosolic ion activities (e.g., Ca^{2+} homeostasis) as well as ion-dependent signaling pathways (e.g., inhibition of Ca^{2+}-dependent enzymes such as phospholipase C) which would ultimately reflect in any of the physiological and morphological changes described above. This is an interesting and potentially important research area regarding mechanisms of Al toxicity, and although there is some evidence in support of an association of Al-induced root growth inhibition with changes in a complex network of responses involving a signal transduction root cells, this is still a topic that is poorly understood and requires more research. For a recent review on Al disruption of Ca^{2+} homeostasis, see Rengel and Zhang (2003).

Oxidative Stress. Reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide that result from photosynthesis and oxidative metabolism can be involved in a number of stress responses (Bowler et al., 1992; Foyer et al., 1994). It has been shown that Al exposure is associated with peroxidative damage of membrane lipids due to the stress-related increase in the production of highly toxic oxygen free radicals (Cakmak and Horst, 1991). However, it appears that lipid peroxidation is only enhanced after a prolonged exposure to Al (24 h or more). Thus, although Al-induced lipid peroxidation does not occur rapidly enough to be an initial mechanism of Al toxicity (Horst et al., 1992; Yamamoto et al., 2001), Al-induced ROS generation and associated mitochondrial dysfunction could still play a more general role in Al inhibition of root growth (Yamamoto et al., 2002).

(D) The Root Cytoskeleton. Because of the central importance of cytoskeletal components (microtubules and microfilaments) in cell division and expansion of a growing root, several laboratories have investigated the cytoskeleton as a potential cytosolic target for Al toxicity. Al could disrupt cytoskeletal dynamics either via a direct interaction with cytoskeletal elements (i.e., microtubules and actin filaments) or indirectly, via alteration of signaling cascades such as cytosolic Ca^{2+} levels that are involved in cytoskeletal stabilization. The orientation of the cytoskeleton provides a template both for cell division and cell-wall biosynthesis (Sivaguru et al., 1999). For example, cortical microtubules are involved in the orientation of cellulose microfibrils, and as such, proper orientation of micotubules is a prerequisite for normal cell expansion. It has been well documented that Al exposure can disrupt both the organization of microtubules and microfilaments in root cells (Alessa and Oliveira, 2001; Blancaflor et al., 1998; Frantzios et al., 2000, 2001; Grabski et al., 1998; Horst et al., 1999; Sasaki et al., 1997a, b; Schwarzerova et al., 2002; Sivaguru et al., 1999, 2003b). For example, exposure to Al results in the disruption and reorganization of cortical microtubules. Likewise, Al induced a significant increase in the tension of the actin filaments of soybean (*Glycine max*) cells (Grabski and Schindler, 1995). Such Al-induced cellular structural changes are likely to result in, and underlie, the morphological changes and structural malformations observed in Al-stressed roots.

(E) DNA/nuclei. Prolonged exposures can lead to Al interactions with structures within the

nucleus, detrimentally affecting DNA composition, chromatin structure, and template activity (Matsumoto, 1991; Sampson et al., 1965; Silva et al., 2000). The presence of Al at the surface of the nucleus can potentially lead to microtubule binding at the membrane surface during the G2 phase of the cell cycle, as well as protein recognition, binding, and transport into the nucleus (Franklin and Cande, 1999; Smith and Raikhel, 1999). These types of interactions of Al with the nucleus can result in the disruption of the cytoskeleton and cell division processes.

The above putative mechanisms of Al toxieity are summarized in the model shown in Figure 1 (left side) except for Al interactions with the cell wall that were not included for reasons of visual clarity.

Physiological mechanisms of Al resistance

Research from a number of laboratories has made it clear that Al resistance can either be mediated via exclusion of Al from the root apex or via intracellular tolerance of Al transported into the plant symplasm. There has been considerable evidence presented in the literature for an Al-exclusion mechanism based on carboxylate exudation from the root apex. More recently, evidence has been presented for an internal tolerance mechanism based on chelation and detoxification of Al in the symplast with carboxylate anions. A summary and overview of both types of resistance mechanisms is considered here, as well as speculation about other possible Al-resistance mechanisms. For recent reviews of this topic, the reader is directed to Barcelo and Poschenrieder (2002), Garvin and Carver (2003), Kochian et al. (2004), Kochian and Jones (1997), Ma and Furukawa (2003), Ma et al. (2001), Ma (2000) and Matsumoto (2000).

Al Exclusion via Root Carboxylate Exudation

The first evidence in the literature for this resistance mechanism came from Miyasaka et al. (1991) who showed in long-term studies that an Al-resistant cultivar of snapbean (*Phaseolus vulgaris)* excreted eight-fold more citrate from the roots than did an Al-sensitive genotype. Citrate is a very potent chelator of Al^{3+}, and it appears that roots do not take up Al-carboxylate complexes. This is supported by the observations in wheat (*Triticum aestivum)* that Al-resistant genotypes release malate, and accumulate significantly less Al in the root apex (but not the mature root regions) compared with Al-sensitive genotypes (Delhaize et al., 1993a; Rincon and Gonzales, 1992; Tice et al., 1992). The seminal work on this resistance mechanism came from Delhaize and Ryan and coworkers who showed, using near isogenic lines (NIL) of wheat differing at a single Al-resistance locus: Al very rapidly activates malate release (within minutes); Al-activated malate release is localized very specifically to the first few millimeters of the root apex of the tolerant NIL (Delhaize et al., 1993a, b; Ryan et al., 1995a, b). Since these initial reports, high levels of Al-activated release of carboxylates have been correlated with Al resistance in a large number of plant species, as summarized in Table 1. When all the evidence in support of Al-activated root carboxylate release as a major resistance mechanism is examined, a very strong case in support of this concept is seen. Some of the major aspects of this resistance mechanism include:

- A correlation between Al resistance and Al-activated carboxylate release in many plant species (Table 1);
- Al-carboxylate complexes are not transported into roots or across membranes (Akeson and Munns, 1990; Shi and Haug, 1990);
- Al resistance cosegregates with Al-induced malate release in wheat and *Arabidopsis* (Delhaize et al., 1993a, b; Hoekenga et al., 2003);
- Activation of carboxyle release is triggered specifically by exogenous $A1^{3+}$ (Ryan et al., 1995a) (although some lanthanide cations can act as $A1^{3+}$ analogs in this response; see Kataoka et al. 2002);
- The rates of Al-activated carboxyate release are dose-dependent on the Al activity in the rhizosphere (Delhaize et al., 1993b; Ma et al., 1997a; Piñeros et al., 2002);
- Overexpression of genes encoding enzymes involved in organic acid synthesis, such as citrate synthase and malate dehydrogenase can, in some cases, result in enhanced Al resistance (de la Fuente et al., 1997; Koyama et al., 2000; Tesfaye et al., 2001);

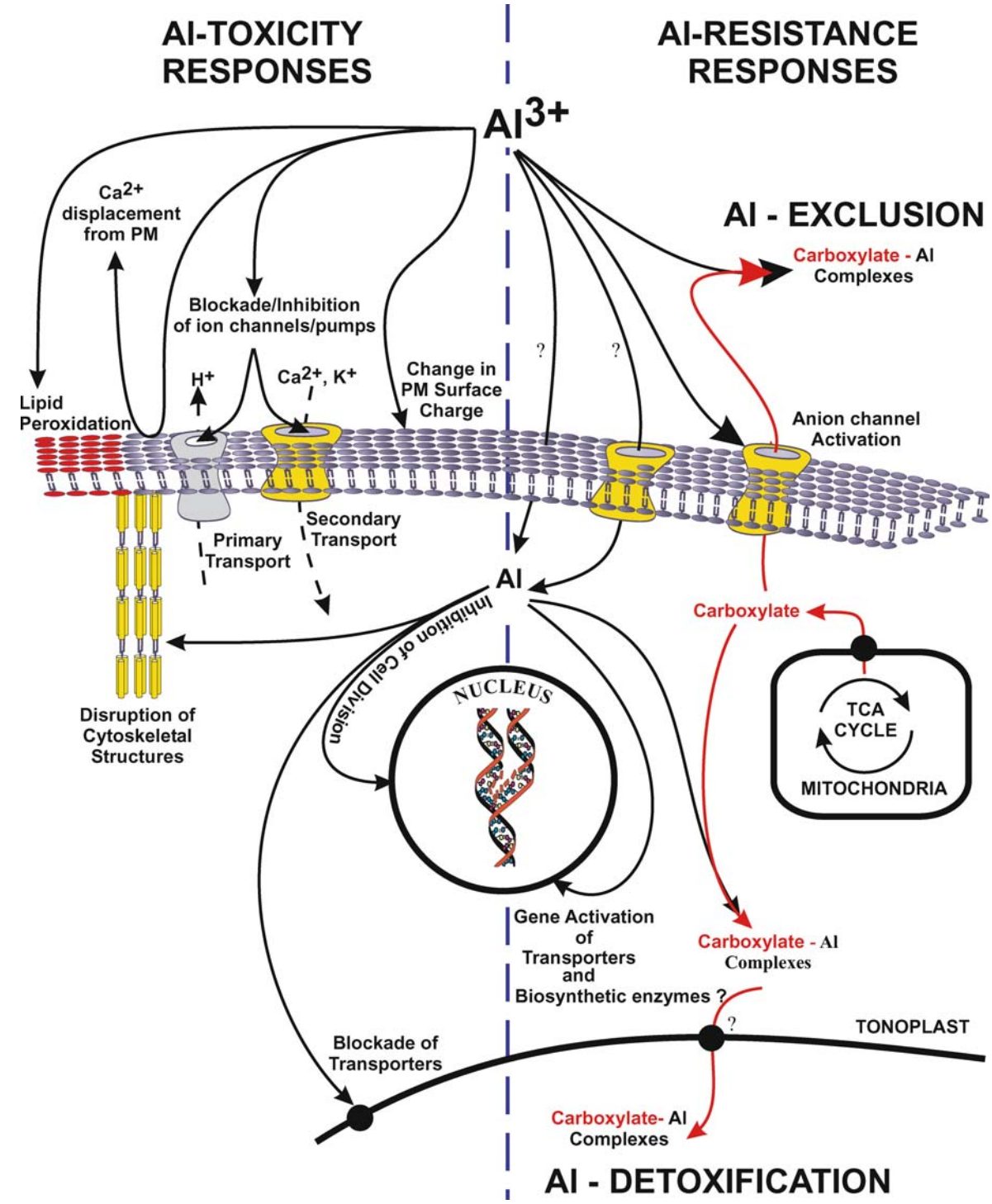

Figure 1. Possible mechanisms of Al toxicity and Al resistance in plants. Al toxicity targets described in the text are illustrated on the left side of the diagram. For clarity, the interactions of Al with the cell wall were not shown. On the right side, Al-resistance mechanisms (Al exclusion and internal Al detoxification) are based on the formation of Al complexes with carboxylates. The Al-exclusion mechanism involves the release of carboxylate anions via an Al-gated anion channel at the plasma membrane. The internal Al-detoxification mechanism involves chelation of cytosolic Al by carboxylate anions with the subsequent sequestration into the vacuole via unknown transporters.

Table 1. Plant species exhibiting Al-activated root carboxylate exudation that is correlated with Al resistance.

Organic Acid Released	Plant Species (common name)	Genotype	Reference
Citrate	*Cassia tora* (sickle senna)		Ishikawa et al. 2000; Ma et al. 1997a
Citrate	*Galium saxatile* (heath bedstraw)		Schöttelndreier et al. 2001
Citrate	*Glycine max* (soybean)	PI 416937	Silva et al. 2001
Citrate	*Glycine max* (soybean)	Suzunari	Yang et al. 2000
Citrate	*Miscanthus sinensis* and *Miscanthus sacchariflorus*		Kayama 2001
Citrate	*Nicotiana tabacum* (tobacco)		Delhaize et al. 2001
Citrate	*Oryza sativa* (rice)		Ishikawa et al. 2000; Ma et al.2002a
Citrate	*Sorghum bicolor* (sorghum)	SC283 and derived NILs	Magalhaes 2002
Citrate	*Zea mays* (maize)	ATP-Y	Kollmeier et al. 2001
Citrate	*Zea mays* (maize)	Cateto-Colombia	Piñeros et al. 2002
Citrate	*Zea mays* (maize)	DK789	Ishikawa et al. 2000
Citrate	*Zea mays* (maize)	IAC-TAIUBA	Jorge and Arruda 1997
Citrate	*Zea mays* (maize)	SA3	Pellet et al. 1995
Citrate, & Malate	*Avena sativa* (oat)		Zheng et al. 1998a
Citrate, &Malate	*Brassica napus* (rape)		Zheng et al. 1998a
Citrate, & Malate	*Helianthus annuus*(sunflower)	Saber et al. 1999	
Citrate, & Malate	*Raphanus sativus* (radish)		Zheng et al. 1998a
Citrate, & Malate	*Secale cereale* (rye)		Li et al. 2000
Citrate, & Malate	*Triticale ssp* (triticale)		Ma et al. 2000
Citrate, & Oxalate	*Zea mays* (maize)	Sikuani	Kidd et al. 2001
Malate	*Arabidopsis thaliana*	Landsberg erecta, Columbia, derived RILs	Hoekenga et al. 2003
Malate	*Triticum aestivum* (wheat)	Atlas 66	Huang et al. 1996; Pellet et al. 1996
Malate	*Triiicum aestivum* (wheat)	Chinese Spring & derived ditelosomic lines	Papernik et al. 2001
Malate	*Triticum aestivum* (wheat)	Kitakami B	Ishikawa et al. 2000
Malate	*Triticum aestivum* (wheat)	Line ET3	Ryan et al. 1995a Delhaize et al. 1993a; Delhaize et al. 1993b
Oxalate	*Colocasia esculenta* (taro)		Ma and Miyasaka 1998
Oxalate	*Fagopyrum esculentum* (buckwheat)		Ma et al. 1997b; Zheng et al. 1998a;b

- An Al-gated anion channel in maize (*Zea mays*) and wheat root tip protoplasts has been identified via electrophysiological experiments, and exhibits the properties necessary for it to be the transporter mediating Al-activated carboxylate release (Piñeros and Kochian, 2001; Piñeros et al., 2002; Ryan et al., 1997; Zhang et al., 2001).

An interesting possible feature of this mechanism is whether it is inducible at the level of gene expression. A number of researchers have assumed that Al-resistance genes and proteins are inducible by Al exposure, and this has been the impetus for some of the molecular studies discussed later. There appears to be evidence in support of an Al-inducible resistance mechanism in some plant species such as rye (*Secale cereale*), triticale (*Triticale ssp.*), and *Cassia tora* (sickle senna), where a lag in Al-activated carboxylate exudation is seen, and the rate of exudation increases over the first 12–24 hrs of Al exposure (Li et al., 2000; Ma et al., 1997a).

However, in other species exemplified by wheat, root malate exudation is very rapidly activated by Al exposure, and the rate of malate efflux does not appear to increase over time. Therefore, in species like wheat Al apparently activates an already expressed carboxylate transporter, and gene activation does not seem to play a role.

In species where the rate of carboxylate exudation apparently increases with time, it is possible that induction of Al-resistance genes contributes to this increased capacity. However, it is still not clear which part of the ligand-release pathway is being induced. The possibilities include: (1) an increased abundance or activity of a plasma-membrane carboxylate transporter; (2) an increased rate of carboxylate synthesis, driven by an increase in abundance or activity of enzymes involved in carboxylate synthesis; and (3) an increased availability of carboxylate ligands for transport, perhaps through altering internal carboxylate compartmentation within cells. To date, no strong evidence exists for a role of any of the enzymes catalyzing carboxylate synthesis and metabolism (PEP carboxylase, malate dehydrogenase, citrate synthase, isocitrate dehydrogenase) in this inductive response. For example, Al activates an up to ten-fold increase in citrate and malate exudation in rye and triticale (with exudation rates being higher in the Al-resistant cultivars), with little or no change in the *in vitro* activities of PEP carboxylase, isocitrate dehydrogenase, malate dehydrogenase, and citrate synthase in the root tips of both Al-resistant and -sensitive cultivars (Hayes and Ma, 2003; Li et al., 2000).

A point that is often ignored regarding this pattern of increasing rates of carboxylate exudation is that this should result in a measurable increase in Al resistance. It is surprising that this has not been addressed in plant species such as rye, where the noticeable induction and time-dependent increase in carboxylate release has been observed. Research in our laboratory on Al resistance in sorghum (*Sorghum bicolor)* has found that Al-activated root citrate exudation correlates closely with Al resistance between two cultivars differing in Al resistance (Magalhaes, 2002). We have found that in sorghum, longer exposures to Al (5–6 days) result in a significant increase in Al resistance compared with measurements of root growth after 24 to 48 hrs of Al exposure. However, even though Al resistance increases over a 6 day Al exposure in sorghum, the Al-activated root citrate exudation actually exhibits a slight *decrease*, suggesting that some other process is induced to cause this increase in Al resistance.

What is the Carboxylate Transporter?

In many plant species, Al exposure rapidly activates the exudation of carboxylate anions, and the release seems to be specific for one or two carboxylate species from a cytoplasm that contains a number of different carboxylate species. Thus, it seems clear that an important part of this Al-resistance mechanism is the activation of a particular carboxylate transporter that presumably resides in the root-cell plasma membrane. In wheat, Al activates malate release almost instantly, suggesting that transport is the limiting step (Osawa and Matsumoto, 2001; Ryan et al., 1995a). It also appears that in wheat increased carboxylate synthesis is not involved in the malate exudation response, as no differences in root tip malate concentration or in PEP carboxylase or malate dehydrogenase activity in Al-resistant versus sensitive genotypes have been observed, even though Al exposure activates a large and continuous efflux of malate in the Al-resistant genotype (Delhaize et al., 1993b; Ryan et al., 1995a, b).

The thermodynamic conditions for carboxylate transport from the cytosol to the external solution suggest that ion channels could be the primary transporter involved in this resistance response. The organic acids in the cytosol exist primarily as anions, and due to the large negative-inside transmembrane electrical potential in plant cells, there is a very strong gradient directed out of the cell for anions. Thus, an anion channel that opens upon exposure to Al would be sufficient to mediate this transport. Anion channels that are specifically activated by extracellular $A1^{3+}$ have recently been identified using the patch-clamp technique with protoplasts isolated from root tips of Al-resistant wheat (Ryan et al., 1997; Zhang et al., 2001) and maize (Kollmeier et al., 2001; Piñeros and Kochian, 2001; Piñeros et al., 2002). In wheat, Ryan et al. (1997) have shown that Al^{3+} activates an inward Cl^- current (indicative of Cl^- efflux; Cl^- was the

only anion in the patch pipette) across the plasma membrane of wheat root protoplasts from the Al-resistant line; as long as Al was maintained in the external solution, this channel remained open. The transport properties for this channel are similar to that exhibited by intact wheat roots for Al-activated malate release. These researchers have also shown that this anion channel can transport malate, and the channel is more active and open more frequently in the presence of Al in root tip protoplasts from the Al-resistant wheat genotype compared with those from the sensitive one. Taken together, these results suggest this anion channel is involved in wheat Al resistance.

A similar anion channel has been identified in root tip cells from Al-resistant maize where Al-activated root citrate release is correlated with resistance (Piñeros and Kochian, 2001; Piñeros et al., 2002). The most important discovery from these studies was that the anion channel could be activated in isolated plasma-membrane patches, where the anion channel is operating in isolation from cytosolic factors. As shown in Figure 2, both whole cells, from maize root tips, and excised membrane patches isolated in the absence of Al, were electrically quiet. When both the whole cells and isolated membranes were exposed to extracellular Al^{3+}, the inward anion current indicative of anion efflux was activated. It was

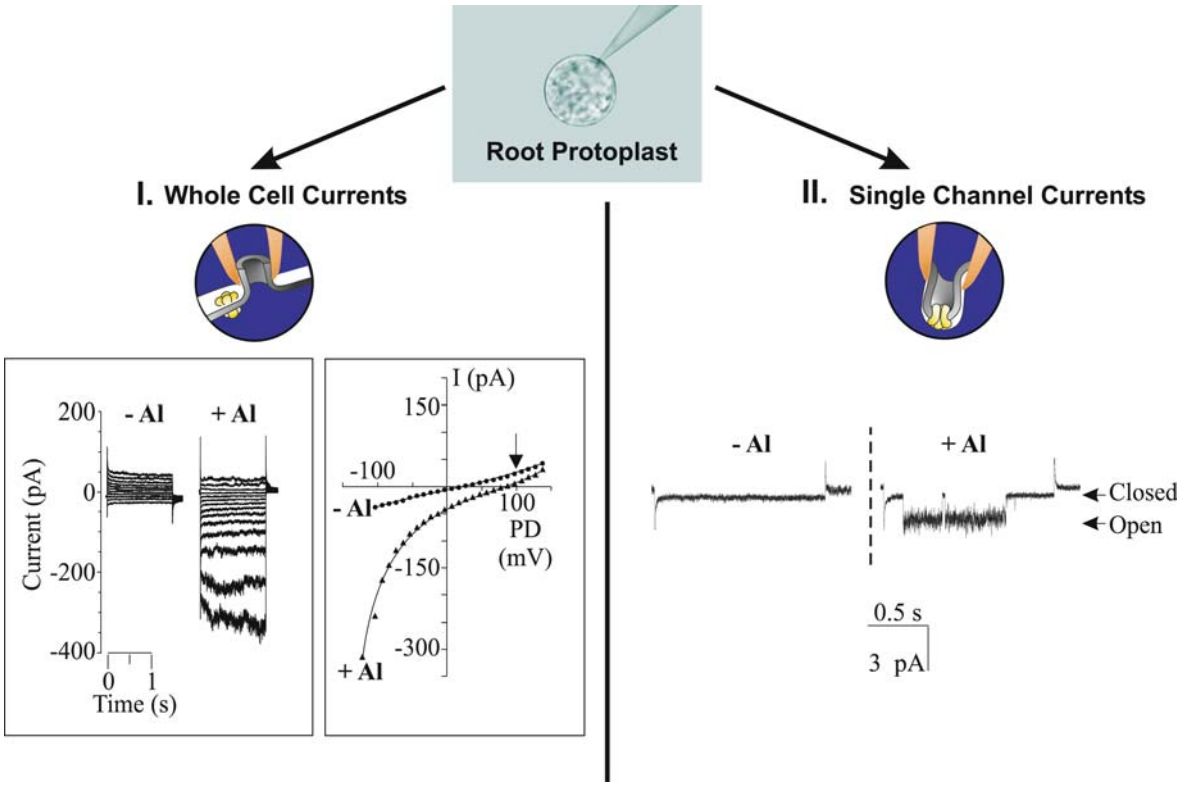

Figure 2. Al-activated anion channel in the plasma membrane of root cells from Al-resistant maize. The patch-clamp technique was employed to record macroscopic currents (whole cell currents on the left) or to study the transporter activity in isolated membrane patches (single channel currents on the right). I. Al activates an inward plasma membrane whole cell anion current (anion efflux). Whole-cell currents were elicited at holding potentials clamped in 10 mV increments. The bath contained 1 mM Cl^- (pH 4.0) minus (left traces) or plus (right traces) 50 μM Al^{3+}. Right panel: Current-voltage relationship for the currents shown on the left. The arrow indicates the Cl^- theoretical reversal potential. II. Al can activate single anion channels in excised membrane patches. A negative voltage potential was employed to test for single-channel activity in outside-out membrane patches excised in the absence of extracellular Al^{3+} (the resulting trace is shown on the left side in II. Subsequently the membrane patch was exposed to extracellular Al^{3+} (50 μM), and the same voltage protocol was employed. The single trace is shown on the right panel. Arrows on the right indicate the closed and open states of the channel.

not determined whether the anion channel could also transport citrate or other organic acids, as Cl^- was used as the primary anion. However, in another study on Al-activated anion channels in protoplasts from root tips of a different Al-resistant line, an Al-activated anion channel could mediate the transport of Cl^-, malate^{2-}, and citrate^{3-} (Kollmeier et al., 2001). Thus, the features needed for Al activation of the anion channel are either contained within the channel protein itself, or are close by in the membrane (e.g., an associated membrane receptor). As depicted in the model summarizing Al resistance mechanisms (Figure 1), and described in detail in a previous review of root carboxylate exudation by Ryan et al. (2001), there are three possible ways that Al could activate a plasma-membrane anion channel involved in carboxylate exudation: (1) Al^{3+} might directly bind to and activate the channel; (2) Al^{3+} might bind to a separate but closely associated membrane receptor, which in turn, activates the channel; or (3) Al^{3+} activates the channel indirectly through a signal cascade that could involve cytosolic components. The findings in Al-resistant maize suggest possibility 1 or 2 as the most likely scenario.

Several families of anion channels have been identified in plants and other organisms, with much of the work coming from animal studies. The two most prominent families include the CLC (Cl channel) family, and a subset of the ATP-binding cassette (ABC) protein superfamily (Barbier-Brygoo et al., 2000). ABC proteins comprise a very large family of transporters that bind ATP during the transport of a wide range of organic and inorganic solutes. One subgroup of ABC transporters includes the cystic fibrosis transmembrane regulator (CFTR) in mammalian cell membranes which has been shown to be a Cl^- channel (Anderson et al., 1991). In yeast, another ABC protein, Pdrl2, mediates carboxylate efflux (Piper et al., 1998). There is no strong evidence that the carboxylate transporter involved in Al resistance is a member of the CLC family. However, there is some circumstantial evidence suggesting it could be an ABC transporter. The anion channel characterized in Al resistant wheat and maize shares a number of transport similarities with an anion channel in the guard-cell plasma membrane, the 'slow' anion channel (for slow inactivation), which also mediates the sustained release of anions (Leonhardt et al., 1999; Schroeder et al., 1993). It has been suggested that the slow anion channel in guard cells could be a member of the ABC family, based on its sensitivity to the ABC transporter antagonist, diphenylamine-2-carboxylic acid (DPC). In an electrophysiological investigation of the Al-activated anion channel in Al-resistant wheat roots (Zhang et al., 2001), DPC inhibited this anion channel, and also inhibited the Al-activated malate efflux from intact wheat roots.

However, some very recent work suggests that at least in wheat, the Al-activated malate transporter may actually be a novel type of membrane transporter. Research in Matsumoto's laboratory has recently described a wheat gene that appears to encode the root tip Al-activated malate transporter (Sasaki et al., 2003). These researchers have found that this gene is expressed more strongly in the root tip of the Al-resistant near-isogenic line (NIL) compared with the sensitive NIL, and appears to encode a novel membrane protein. Heterologous expression of this gene, named *ALMT1* for Al-activated malate transporter, resulted in Al-activated malate exudation in *Xenopus* oocytes, as well as in roots of transgenic rice (*Oryza sativa)* seedlings and tobacco (*Nicotiana tabacum)* suspension cells. Furthermore, expression of *ALMT1* increased Al resistance in tobacco suspension cells. This finding may represent the identification of the first major Al-resistance gene in plants.

Al resistance mechanism involving internal detoxification

Several researchers have recently identified a second Al resistance mechanism that is based on the complexation and detoxification of Al after it enters the plant. This discovery has come from research focusing on plants that can accumulate Al to high levels in the shoot. For example, *Hydrangea macrophylla* is an ornamental plant whose sepals turn from red to blue when the soil is acidified; this color change is due to Al accumulation in the sepals resulting in the formation of a blue complex of Al with two compounds, delphinidin-3-glucoside and 3-caffeolylquinic acid (Takeda et al., 1985). *Hydrangea macrophylla* can accumulate more than 3000 μg Al g^{-1} dry weight

in its leaves (Ma et al., 1997c); Ma and colleagues showed that the Al in the leaves exists primarily as a 1:1 Al-citrate complex. This type of complex should bind Al very tightly in a cytosol with a pH of around 7, and should protect the cytosol against Al injury. Ma and colleagues also studied a second Al accumulator, buckwheat (*Fagopyrum esculentum*). A portion of the Al resistance in buckwheat is due to Al-activated oxalate exudation from the root apex (exclusion) (Zheng et al., 1998b). However, buckwheat also accumulates Al to very high levels in its leaves, as high as 15, 000 μg Al g^{-1} dry weight when the plant is grown on acid soils (tolerance) (Ma et al., 2001). Most of the Al in both roots and leaves was complexed with oxalate in a 1:3 Al-oxalate complex (Ma et al., 1998). Subsequently, they showed that the Al being transported to the shoot in the xylem sap is complexed with citrate, and not oxalate (Ma and Hiradate, 2000). These findings suggest that the Al undergoes a ligand exchange from oxalate to citrate when it is transported into the xylem, and is exchanged back with oxalate when in the leaves. Leaf compartmental analysis showed that 80% of the Al in buckwheat leaves was stored in vacuoles as a 1:3 Al-oxalate complex (Shen et al., 2002). On the right side of Figure 1, where the different possible Al-resistance mechanisms are depicted, this internal detoxification mechanism is shown to involve Al chelation in the cytosol and subsequent storage of the Al-carboxylate complex in the vacuole. The tonoplast-localized mechanisms mediating the transport of Al into the vacuole, as well as the nature of its substrate (i.e., free Al versus Al-carboxylate complexes) remain unknown.

Genetic and molecular aspects of aluminum resistance

Increasing Al resistance has been a goal for plant scientists for many years, as this should lead to increased crop production on acid soils (see Garvin and Carver, 2003; Hede et al., 2001, for recent reviews). The majority of plant breeding attention has been focused on the economically important grasses (e.g., wheat, rice, maize), and thus most of the studies on the inheritance of Al resistance have been performed in these species. In the Triticeae, Al resistance has relatively simple, or qualitative, inheritance, such that one major gene explains the majority of Al resistance observed. By contrast, rice and maize have complex, quantitative inheritance of Al resistance, such that several genes are required to explain a plurality of Al resistance differences. More recently, genetic experiments have been conducted in model plants, such as *Arabidopsis thaliana*, to identify genes important for Al resistance. In spite of this body of work, Al-resistance genes have yet to be cloned from any species, with the exception *of ALMT1* from wheat (Sasaki et al., 2004). This situation is sure to change in the coming years, with the utilization of genome sequence analyses from rice and *Arabidopsis* and the application of genomics-based approaches to gene discovery.

Genetics of Al resistance: cases of simple inheritance

The inheritance of Al resistance in wheat (*Triticum aestivum*) has been studied longer and, perhaps, more completely than in any other plant species. Crop improvement programs in Brazil and the US led to the development of excellent cultivars (cv. BH1146 and Atlas66, respectively), which have subsequently been well studied in both field and laboratory conditions. In many crosses between these elite Al-resistant cultivars and Al-sensitive varieties, Al resistance is apparently conferred by a single, dominant locus; in other crosses, segregation patterns suggest two loci are responsible for resistance (Garvin and Carver, 2003). One of these loci has been mapped to the long-arm of chromosome 4D, sometimes called Alt_{BH} or *Alt2* (Milla and Gustafson, 2001). The existence of other loci important for Al resistance elsewhere in the genome can further be inferred from the study of varieties that contain chromosomal deletions. Additional locations have been identified through the comparison of nullisomics, ditelosomics or lines with small segmental deletions that have diminished resistance to Al relative to their euploid progenitors (Aniol and Gustafson, 1984; McKendry et al., 1996; Papernik et al., 2001). One can imagine that most cellular processes will have multiple components, such that mutations or deletions in any single part could compromise the entire mechanism. The

importance of these additional genetic factors beyond those located on 4DL (e.g., 5 AS, 7AS) have been difficult to evaluate, as genetic experiments have yet to implicate these regions as containing resistance genes by cosegregation analysis.

It has been well established that Al resistance in wheat is highly correlated with an Al-activated release of malate. In a seminal study, Ryan et al. (1995b) quantified both relative root length and malate release in thirty-six cultivars to assess Al resistance. A correlation analysis of these two parameters demonstrated that 84% of the variance was explained such that differences observed in relative root length (RRL) were largely explained by the quantity of malate released. This high degree of agreement suggests that differences within a single physiological mechanism are responsible for the majority of differences in Al resistance between wheat cultivars. Taken together with the observations regarding cosegregation analysis, it is possible that a very few regulatory loci are responsible for the differences in Al resistance reported in wheat. This is not to say that malate release is the only Al-resistance mechanism in wheat; merely that malate release is only Al-resistance pathway to exhibit polymorphism among wheat cultivars studied to date.

Rye (*Secale cereale*) is generally regarded as having excellent resistance to abiotic stressors, including Al, superior to that observed in its close relative wheat (Aniol and Gustafson, 1984). Unlike wheat, rye is self-incompatible, and thus an obligate out-crossing species. This may help explain why cosegregation experiments in rye generally detect a greater number of Al resistance loci than are detected in wheat (Aniol and Gustafson, 1984; Gallego and Benito, 1997; Hede et al., 2001), Like wheat, the long-arm of chromosome 4 contains a major Al-resistance locus, called *Alt3* (Gallego and Benito, 1997). An improved map estimate for *Alt3* demonstrated tight linkage with markers linked to Alt_{BH}, advancing the suggestion that homeologous loci act as Al resistance genes in both species (Miftahudin et al., 2002). A second resistance locus (*Alt1*) has been mapped to a small interval on the short-arm of chromosome 6 (Gallego et al., 1998). Unfortunately, the molecular markers closely linked to *Alt1* have not been used for additional mapping studies or otherwise incorporated into a consensus rye genetic map, such that fine-scale comparative mapping is not possible.

Barley (*Hordeum vulgare*), a third member of the Triticeae tribe, also contains a major Al resistance locus, *Alp*, on the long-arm of chromosome 4 (Minella and Sorrells, 1992). Like *Alt3* in rye, the *Alp* locus is linked to markers useful for following Alt_{BH} in wheat (Tang et al., 2000) (Figure 3). Unlike rye or wheat, barley is very sensitive to Al (Minella and Sorrells, 1992). If *Alp, Alt3* and Alt_{BH} were truly orthologous loci, one would expect that an analysis of the protein sequences should reveal a great deal about how this wide range of tolerance phenotypes is achieved.

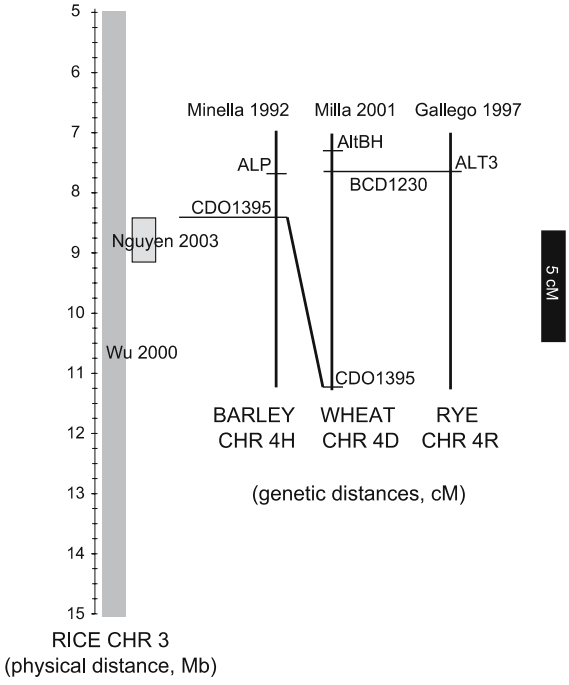

Figure 3. Comparative mapping of Al-resistance loci between Triticeae and rice. Loci located to rice linkage block 3C. Flanking genetic markers for the QTLs identified in rice (Nguyen et al., 2003; Wu et al., 2000) were located on the rice chromosome 3 pseudo-molecule using Gramene. The CDO1395 marker defined one QTL boundary (Nguyen et al., 2003) and served to link this region of the rice genome to the corresponding region in genomes of species in the Triticeae, Genetic distances between linked markers and Al-resistance genes in the Triticeae (Gallego et al., 1997; Milla et al., 2001; Minella et al., 1992) are shown. The 5 cM genetic distance scale bar was set using the physical to genetic distance ratio from rice over this interval (approx. 0.36 Mb/1 cM).

Al resistance genetics in sorghum (*Sorghum bicolor L.* Moench) has only very recently received any attention. Sorghum is closely related to maize, possesses the second smallest genome among cultivated grasses (after rice), and exhibits a wide phenotypic range for biotic and abiotic stress resistance making this warm-weather grass an attractive model experimental system (Mullet et al., 2001), Recent investigations into the inheritance of Al resistance indicate that, like wheat, rye and barley, sorghum exhibits a simple pattern of inheritance with a single locus explaining the majority of differences observed among genotypes (Magalhaes, 2002; Magalhaes et al., 2003). Unlike the Triticeae, the Alt_{SB} locus in sorghum is neither located in the homeologous chromosomal location to *Alp, Alt3* and Alt_{BH}, nor is it linked to the shared set of RFLPs and SSRs. The phenomenology of Al resistance is also somewhat different in sorghum, relative to the Triticeae, as the resistance response appears to be inducible and take days to fully manifest (Magalhaes, 2002). The combination of genetic and physiological data suggests that sorghum utilizes a different pathway to achieve Al resistance than the mechanism characterized in wheat and its relatives.

Genetics of Al Resistance: Cases of Complex Inheritance

Rice (*Oryza sativa)* has been the subject of the largest number of quantitative trait locus (QTL) mapping experiments to identify the basis of Al resistance (Ma et al., 2002a; Nguyen et al., 2001, 2002, 2003; Wu et al., 2000). These studies used ten different parents, including improved *indica* and *japonica* cultivars, and a wild relative, *Oryza rufipogon*. Twenty-seven QTLs important for Al resistance, as estimated by relative root growth, were identified in the five studies. Given the conservation of location for Al resistance loci among the Triticeae, one wonders if an ortho-logous locus to $Alp/Alt3/Alt_{BH}$ plays a similar role in rice. Although a portion of rice chromosome 3 (rice linkage block 3C) is homeologous to Triticeae 4L (Gale and Devos, 1998), and genetic markers linked to Al-resistance loci are shared between rice, wheat, and barley (Figure 3) (Nguyen et al., 2003), the Al resistance locus on rice 3 is not the primary one for resistance in rice. Instead, a locus on rice 1 typically explains the largest percentage of Al resistance in the mapping populations studied. Rice 1 was identified by all five studies as important for Al resistance (Figure 4A). Interestingly, the rice 1 QTL is in a region homeologous (rice linkage block 1B) to the portion of sorghum linkage group G that contains Alt_{SB} (Figure 4A). Further work will be necessary to evaluate whether orthologous loci are at work in both sorghum and rice, or if the apparent linkage is merely serendipitous.

Two other chromosomal regions were repeatedly identified among rice mapping populations as important for Al resistance. An interval on rice 9 was identified by three studies; in each case, the *indica* parent provided the sensitive allele (Figure 5A) (Nguyen et al., 2002, 2003; Wu et al., 2000). The indica parent again provided the sensitive alleles for a common QTL on rice 8, although this interval was identified by only two studies (Figure 5B) (Nguyen et al., 2002, 2003).

Like wheat, maize (*Zea mays* spp. *mays*) has long been the subject of breeding programs that seek to increase Al resistance or understand the basis for it (Magnavaca et al., 1987; Sawazaki and Furlani, 1987). Some investigators concluded that Al resistance was a qualitative trait, although these studies utilized either small mapping populations (<100 F2 individuals) or nearly identical mapping parents (a resistant inbred and a sensitive somaclonal variant) (Rhue et al., 1978; Sibov et al., 1999). The majority of investigators have concluded that Al resistance is a quantitative trait, based on the segregation analysis of large F2:3 populations or recombinant inbred lines (Giaveno et al., 2001; Magnavaca et al., 1987; Ninamango-Cardenas et al., 2003; Sawazaki and Furlani, 1987). Only one QTL mapping study has been published to date; five genomic regions were identified as important for Al resistance (Ninamango-Cardenas et al., 2003). When we conducted an *in silico* comparative mapping analysis of these regions using the Gramene database (Ware et al., 2002), markers flanking two of these regions in maize could be located to regions containing Al resistance loci in other grasses. The markers that flank a principal maize QTL region (bin 6.05) can be landed to rice chromosome 5, in the same vicinity of a QTL identified by Nguyen et al. (2001) (Figure 4B). Perhaps more intriguing is the fact that the third most important QTL from the maize

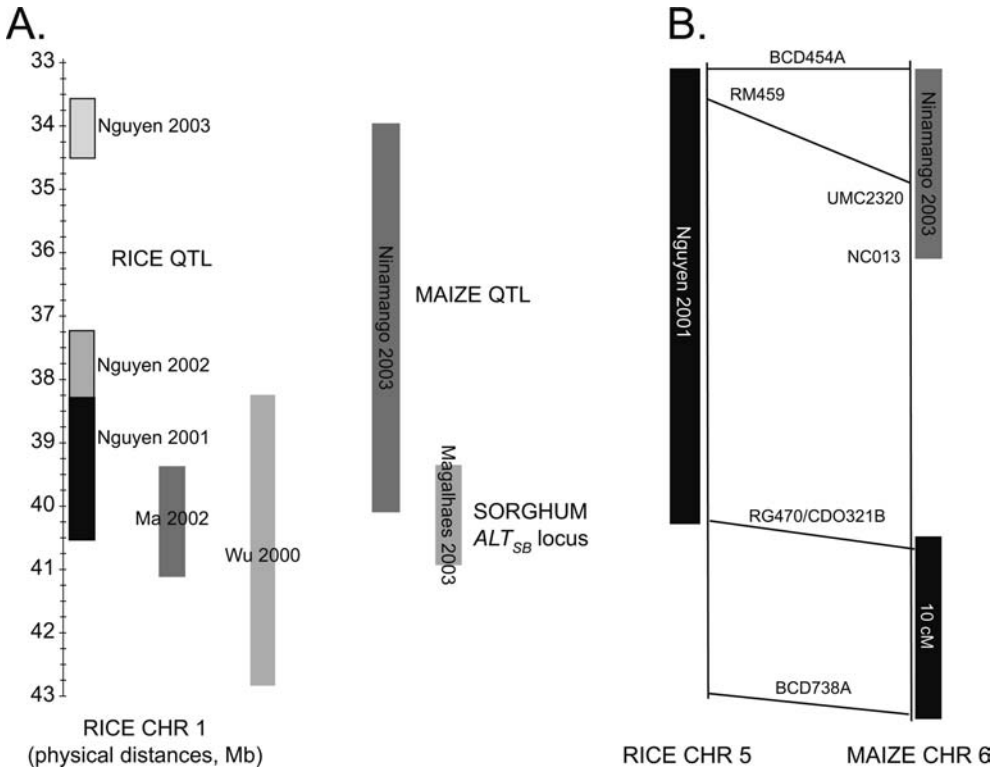

Figure 4. Comparative mapping of Al-resistance loci between rice, maize and sorghum. A) Loci located to rice linkage block 1B. Fanking genetic markers for the QTLs identified in rice (Ma et al., 2002a; Nguyen et al., 2001, 2002, 2003; Wu et al., 2000), maize (Ninamango-Cardenas et al., 2003), and sorghum (Magalhaes et al., 2003) were located on the rice chromosome 1 pseudomolecule using Gramene. The gray bars indicate QTL confidence intervals; distances are in Mb. B) Loci located to rice linkage block 5B. The BCD454A marker defined one boundary for a QTL in rice (Nguyen et al., 2001) and mapped very close to the boundary for a maize QTL (Ninamango-Cardenas et al., 2003). Three additional markers mapped in both rice and maize over this region are shown to demonstrate the extent of synteny.

study (bin 8.07) falls within a region of the maize genome homeologous to rice chromosome 1 and sorghum linkage group G, the chromosomal segment identified by six different studies (Figure 4A). As the physical map of maize improves, it should be possible to locate the other maize QTLs to their related chromosomal regions in rice, to identify possible orthologous tolerance loci. In any event, there is a great deal of exciting comparative mapping to be done in the grasses, to investigate the role of putative orthologs in Al resistance.

Two studies offer insight into the basis for Al resistance in the model species *Arabidopsis thaliana* (Hoekenga et al., 2003; Kobayashi and Koyama, 2002). This pair of studies utilized the same Lands-berg *erecta* X Columbia RIL mapping population. In both studies, the principal QTL was found at the top of chromosome 1 and explained approximately 30% of the variance observed; however, the locations for all of the other putative QTLs were in complete disagreement between studies. This outcome is likely due to the affect of the growth conditions on root growth in each study. Kobayashi and Koyama (2002) used a low ionic strength hydroponic growth condition at pH 5.0, while Hoekenga et al. (2003) used higher ionic strength nutrient solution and Al concentration in gelled (semi-solid) growth media. As any phenotype is the result of both genetic and environmental effects, the differences in growth conditions likely illuminated factors important for the resistance of low ionic strength and/or pH, in addition to the intended target of Al tolerance.

Molecular Biology of Al Resistance

Genomics-based inquires are, by their nature, multidisciplinary endeavors that integrate several lines of inquiry. Based on the physiological char-

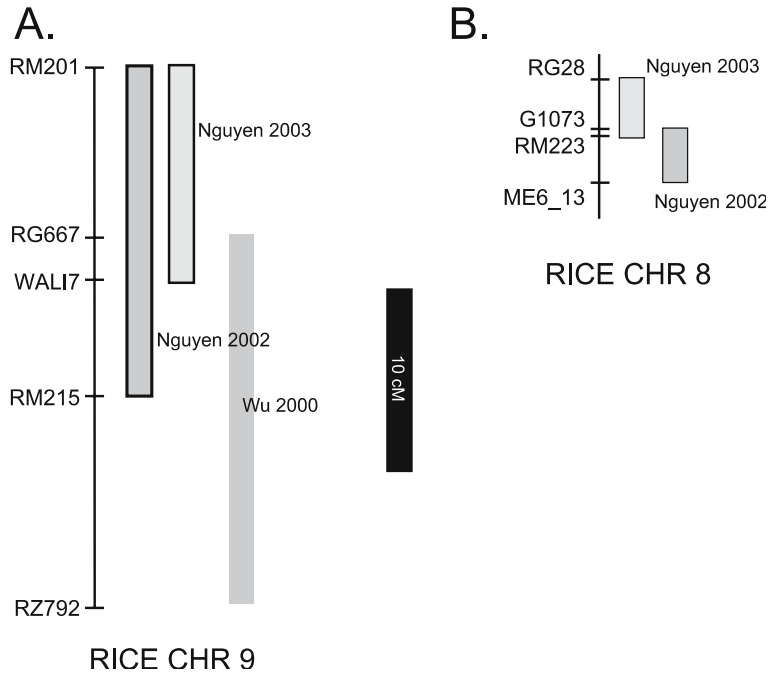

Figure 5. Comparative mapping of Al-resistance loci among rice varieties. The gray bars indicate QTL confidence intervals; distances are in cM, where the scale is shared in both panels. (A) Loci located to rice linkage block 9. Three rice QTLs were identified in the same interval of rice chromosome 9 (Nguyen et al., 2002, 2003; Wu et al., 2000). (B) Loci located to rice linkage block 8. Two rice QTLs were identified in the same interval of rice chromosome 9 (Nguyen et al., 2002, 2003).

acterizations of Al resistance and toxicity, it is clear that carboxylate release and internal detoxification are key mechanisms in Al resistance. This prior knowledge facilitates the analysis of large-scale gene ('microarrays') or protein ('proteomics') expression datasets, as candidate Al-resistance factors should make themselves obvious from the collected data. Furthermore, Al resistance may be an inducible process, (e.g., Li et al., 2000; Magalhaes, 2002), such that profiling gene or protein expression through a time course of Al toxicity may be useful. Unfortunately, the modern techniques of microarray or proteomic analysis have yet to be fully applied to Al resistance questions; one can assume this will change in the near future.

Gene-expression profiling

The primary strategy employed to estimate genome-wide changes in gene expression during Al stress has been to construct subtractive cDNA libraries. Libraries have been constructed from sugarcane Saccharum spp. (hybrid cv. N19), tobacco, *Arabidopsis*, rye and wheat to identify genes more highly expressed in Al-stressed roots (Drummond et al., 2001; Ezaki et al., 1995; 1996; Milla et al., 2002; Richards et al., 1998; Watt, 2003). Candidate genes identified in this manner are then typically assessed using transgenic plants; transformants are subsequently challenged with Al, and their resistance evaluated (Ezaki et al., 2000, 2001; Sivaguru et al., 2003a). Ezaki et al. (2000) had only modest success in increasing the Al resistance of *Arabidopsis* with their transgenes, with gains of 50% or less (from 60% inhibition of root growth in untransformed to 40% inhibition in transgenics). An-ionic peroxidase from tobacco produced the largest and most consistent increase in Al resistance in these experiments, presumably by increasing the capacity of the plant to cope with reactive oxygen species. Basu et al. (2001) produced a similar result working in *Brassica napus*, where they over-expressed a manganese superoxide dismutase and observed a modest gain in Al resistance. These results reinforced the importance of free radical quenching as an Al-resistance mechanism. However, truly novel

mechanisms of Al resistance have yet to be identified using this strategy.

Only one microarray experiment has been published on the effects of Al stress on gene expression (Hoekenga et al., 2003). This experiment was limited in scope, due to the fact it was conducted with the *Arabidopsis* Functional Genomics Consortium (AFGC), utilizing an 8000 feature array and a single, replicated hybridization. One of the benefits of the (now defunct) AFGC program is that all of the data generated are searchable via The *Arabidopsis* Information Resource (TAIR) website (Finkelstei et al., 2002; http://www.Arabidopsis.org). As these data were up-loaded to the public website shortly after the microarray experiments were complete, other workers in the Al resistance field gained access to the gene expression profiling data. In this manner, Schultz et al. (2002) were able to identify that the Al treatment differentially affected members of the arabinogalactan protein (AGP) family, which is their study area. AGPs are cell-wall localized proteins thought to be important for growth and development, but poorly characterized. Al stress induced two members and repressed two others of the nineteen-member gene family. AGP2 was demonstrated to be Al inducible using Northern Blot hybridization, reaching maximal expression after 8 h of exposure (Schultz et al., 2002). Thus, a novel facet of Al-stress response was identified, although it is still unknown what role AGP2 does play in modulating cell wall architecture or structure.

Concluding remarks

The considerable interest in mechanisms of plant Al resistance and toxicity exhibited by researchers around the world has resulted in an increased understanding of the mechanistic basis for these complex topics. This is particularly true for investigations into the molecular, genetic and physiological mechanisms of Al resistance. Al-resistant genotypes in many plant species appear to employ Al-activated carboxylate release as a resistance mechanism based on exclusion of Al from the growing root tip. Furthermore, we now have a better understanding of many of the physiological features of this resistance mechanism. However the research has also resulted in many questions that remain unanswered. For example, why do some species release malate, others citrate, and still others oxalate in response to Al stress? How do roots perceive the Al signal and transduce this signal to activate the processes involved in resistance? What other Al-resistance mechanisms are employed by different plant species? We are just beginning to understand a second Al-resistance mechanism involving internal detoxification of Al with carboxylate ligands and the sequestration of the Al-carboxylate complexes in the vacuole, and it is likely that other additional resistance mechanisms exist. Finally, we are poised to discover the genes that underlie Al resistance mechanisms. The identification and characterization of Al resistance genes will not only greatly advance our understanding of the mechanistic functioning of these processes, but, more importantly, will be the source of new molecular resources that researchers will use to develop improved crops better suited for cultivation on the acid soils that comprise such a large fraction of the world's lands.

Acknowledgements

The authors want to acknowledge the financial support for their own research on acid soil tolerance from the USDA-CSREES National Research Initiative Competitive Grants Program (USDA-NRI Grants #00–35100–9280, #01–35301–10647, and #02–35100–12058), the McKnight Foundation Collaborative Crop Research Program, and from the USDA Agricultural Research Service.

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